# REGENERATION OF THE ANTERIOR END OF AULOPHORUS FURCATUS (NAIDIDAE) WITH SPECIAL REFERENCE TO EFFECT OF X-RAYS [1, 2]

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*Aulophorus furcatus* is an asexual, aquatic naid 3 to 7 mm. long and has the ability to regenerate very rapidly. The literature, however, reveals only taxonomic studies (Stephenson, 1930; Cernosvitov, 1944). Effects of irradiation on annelid regeneration have been almost entirely limited to total irradiation of varying doses (Stone, 1932, 1933; Turner, 1934, 1935). Zhinkin (1934) grafted lethally irradiated segments to normal hosts and attributed the resulting regeneration in the irradiated graft to a cell type, the neoblast, which had migrated from the healthy host. The neoblast is considered the vital factor by some investigators, while others believe it unimportant (Hämmerling, 1924).

In 1948, a technique of partially shielding an immobilized planarian exposed to x-radiation was reported (Wolff and Dubois, 1948). Their method was modified and applied to *A. furcatus*. Observations of the neoblasts in irradiated and non-irradiated worms were made in an effort to determine whether they contributed to regeneration in this species.

## Materials and Methods

Stock cultures of worms were maintained in glass dishes filled with distilled water and were fed boiled lettuce. Experimental worms were transferred to interlocking castor dishes. Amputations of the anterior ends were made at the sixth segment just posterior to the pharynx (Fig. 1). In the anteriorly irradiated worms, at least 8 and usually 12 to 15 segments were present between the wound and the shielded part of the worm.

The animals which had only the anterior half irradiated were immobilized by covering them with a thin layer of 2% agar. The agar containing the worms was cut into blocks. These were aligned on a watch glass and a three-mm. thick lead strip was placed over the posterior halves of the worms. They were then exposed to the determined lethal dosage of 2600 r at a rate of 300 r per minute. The radiation was produced by a 200 Kv air-cooled tube. A cardboard filter was used to minimize heat. Amputations of the head were made immediately after exposure.

[1] This investigation was carried out under direction of Dr. H. W. Beams, in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Zoology, The State University of Iowa, Iowa City, Iowa.

[2] Irradiation by permission and under direction of Dr. T. C. Evans, Radiation Research Laboratory, The State University of Iowa, Iowa City, Iowa.

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Three series of worms were observed in detail: one non-irradiated, one totally irradiated, and one of anteriorly irradiated worms. Totally irradiated animals died 14 to 21 days after exposure to 2600 r. Normal regeneration was completed during the fifth day. At selected intervals following amputation, the worms were fixed in Bouin's solution. Selected sagittal sections were stained with Delafield's hematoxylin and eosin Y and microphotographed at 600 diameters.

No grafting experiments were made. In preliminary work, it was observed that this species will discard any injured segment. Healing immediately follows and no fusion would occur. The fixative was also the killing agent as this worm rapidly autolyzes immediately following death. General observations were made on regeneration of the tail of normal and posteriorly irradiated animals.

## Results

The observations on the non-irradiated worms indicated a sequence of recovery similar to that described in other species of the Naididae (Krecker, 1923; Stephenson, 1930). The initial response was an epidermal healing with no mitoses evident (Fig. 4). Simultaneously, the severed end of the alimentary tract closed over. In three to six hours, a migration of the neoblasts was evident (Fig. 3). Next, the epidermal scar thickened and some cell division occurred, after arrival of neoblasts. This thickening proceeded until the epidermal cap was formed, the newly formed cells seemingly pushed inward. These, together with the proliferating endodermal cells of the intestine, formed a column or cord of cells termed the cell strand. Between the dorsal side of the cell strand and the epidermal cap, a cluster of epidermal cells formed a primordial cerebral ganglion which was well developed after 12 hours.

Continued canalization of the cell strand formed the regenerated anterior alimentary tract. The muscular layers of the intestine developed into the thicker walls of the new pharynx. The fate of the neoblasts was not positively determined. That some neoblasts enter into the formation of mesodermal tissues is likely considering the numbers present before cell differentiations had begun. The neural structures apparently developed from existing ganglion cells and the cells of the regenerated cerebral ganglion. An average of five days passed before the regenerated head was used in feeding and locomotion. The appearance of a normal and regenerated head is shown in Figure 1.

In the totally irradiated specimens, no regeneration occurred. An epidermal healing succeeded amputation in two to four hours. The scar thickened more than in non-irradiated worms. Neoblastic activity was never evident. The size of the existing cells became progressively smaller until death resulted (Fig. 2).

The anteriorly irradiated worms had an immediate healing response and neoblasts in the shielded segments were active four to six hours after amputation. There was a delay in the initiation of regeneration of 12 to 24 hours (Figs. 5 and 6). Other than this, regeneration proceeded as in the non-irradiated worms. Some of the irradiated worms shed the cuticle from the exposed segments on the second or third day. Complete morphological recovery required one to two additional days. Functional recovery necessitated two to four days longer than in the non-irradiated worms.

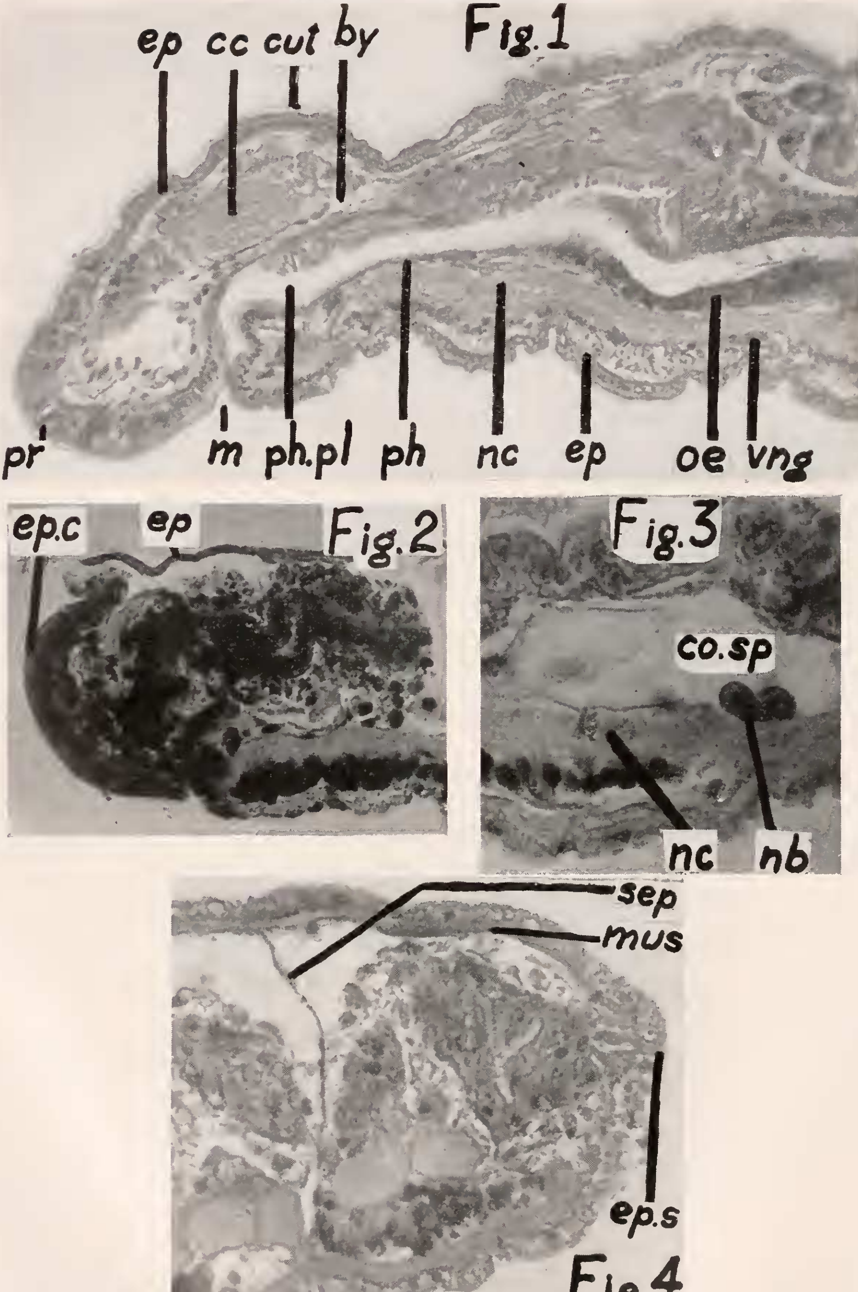
Fig. 1
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FIGURES 1–4.

In posterior regeneration, in both irradiated and non-irradiated worms, the neoblasts were seen migrating to the wound site. No detailed microscopy was made of posterior end regeneration.

## Discussion

The results indicated that the neoblasts, in this species, were involved in the regeneration process, either directly or indirectly. The presence of the neoblasts in the wound area contributed, in some way, to initiation of mitotic activity in the epidermal cap and cell strand. It is probable that the neoblasts form some of the regenerated mesodermal tissues. Several workers, using many neoblastic species, have concluded that the neoblast is essential to regeneration (von Wagner, 1906; Krecker, 1923; Sayles, 1927; Zhinkin, 1934). Contrary arguments as to the importance of neoblasts have been presented by other workers (Hepke, 1897; Abel, 1902). They attributed no function to the neoblast in the regeneration process. In all of these experiments, however, irradiation was not used.

Irradiation experimentation on two annelid species gives support to the contention that neoblasts, when present, do have an important role in regeneration. Irradiation of the neoblastic species, *Tubifex tubifex* (Stone, 1932, 1933) and *Lumbriculus inconstans* (Turner, 1934, 1935), revealed that the regenerative processes were halted or delayed. The conclusion was that the embryonic neoblasts were killed or severely inhibited. Similar experiments and results on species of planaria have been reported (Curtis, 1936). In *Rhynchelmis limosella*, irradiated segments were grafted to a non-irradiated host (Zhinkin, 1934). Regeneration in the graft began after the neoblasts had migrated from the host through the graft to the wound area. Observations on a planarian species in which one-half of the animal was irradiated have been reported (Wolff and Dubois, 1948). It was noted that regeneration in the distal portion of the irradiated half occurred after neoblasts had migrated from the non-irradiated portion to the wound area. No neoblasts were

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FIGURES 1–4.

Abbreviations are as follows:

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|---|---|---|---|
| bv. | blood vessel | m. | mouth |
| cc. | cerebral commissure | mus. | muscle layer of body wall |
| cg. | cerebral ganglion | nb. | neoblast |
| co. sp. | coelomic space | nc. | nerve cord |
| cs. | cell strand | ph. pl. | pharyngeal plate |
| cut. | cuticle | ph. | pharynx |
| ep. s. | epidermal scar | pr. | prostomium |
| ep. c. | epidermal cap | vng. | ventral nerve ganglion |
| ep. | epidermis | sep. | septum |

All sections are sagittal.
Magnification is 600 × except Figure 3 which is 900 ×.

FIGURE 1. Mid-sagittal section of head of normal worm.

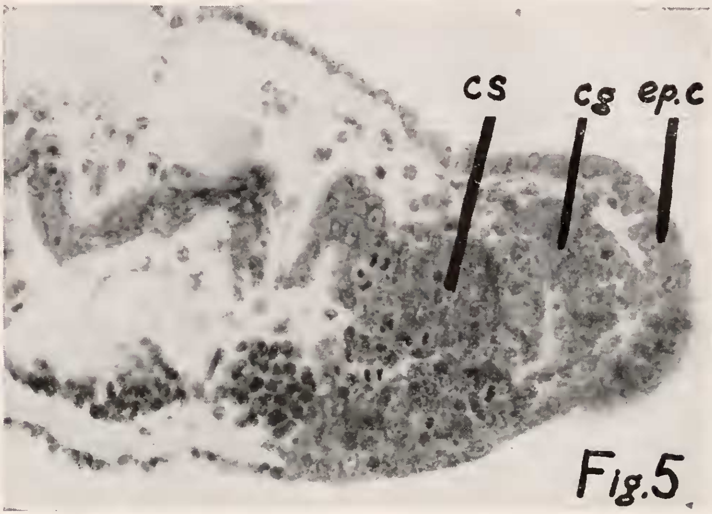
FIGURE 2. Anterior end of specimen 18 days following total irradiation of 2600 r. Head was amputated immediately following exposure. No cell strand. Reduced body diameter, all structures atrophied.

FIGURE 3. Two neoblasts migrating anteriorly along sheath of nerve cord. Large nuclei, prominent nucleolus. Section made of specimen six hours after amputation of head.

FIGURE 4. Four hours after amputation of head showing epidermal healing.

evident in the irradiated half. The most extreme interpretation of the regeneration role of the neoblasts was that of Hämmerling (1924). He compared the neoblasts to the meristem cells of plants or the interstitial cells of coelenterates.

The migratory ability of the neoblasts in *A. furcatus* was more extensive than that reported in other species. Migration toward the posterior end only was re-



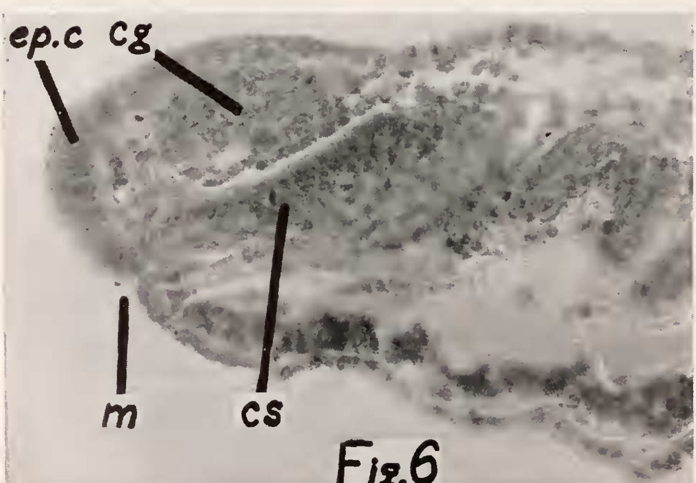




FIGURE 5. Forty-eight hours after head amputation of anteriorly irradiated worm. Many mitoses present. Mouth notch not yet present. Cell strand not as definite as in Figure 4.

FIGURE 6. Forty-eight hours after head amputation of normal worm. Cell strand well developed, mouth notch present. Cerebral ganglion well developed. Mitoses infrequent. Prostomium taking form.

ported in *Lumbriculus* (Sayles, 1927) and a maximum anterior migration of 7 to 9 segments in *Limnodrilus* and *Tubifex* (Krecker, 1923). In *A. furcatus,* anterior migration was observed through 15 or more segments.

The delay in the initiation of regeneration can be explained only in part by the time taken by the migration of the neoblasts. The general effects on other tissues (Clark, 1940; Lea, 1947) also contributed to the delay. Replacement of some cells and recovery from irradiation injury by the tissues, particularly the epidermis and endoderm, were necessary.

The posterior end of the worm is considerably different from that of the majority of oligochaetes. It has three pairs of gill-like processes and a pair of palps extending beyond the anal pore. Details of the histology of this posterior structure are yet to be recorded. Hence, posterior regeneration merited only general observation.

## Summary

1. A comparative study of regeneration of the head of *Aulophorus furcatus* in non-irradiated, anteriorly irradiated and totally irradiated worms has been presented. Totally irradiated worms failed to regenerate a new head. A delay in the initiation of regeneration and rate of regeneration was noted in the worms which had the anterior half exposed to lethal irradiation. The lethal irradiation apparently had its effect by killing or severely inhibiting the neoblasts.

2. In this species, it was demonstrated that the neoblasts are capable of migrating both anteriorly and posteriorly. They are able to migrate into irradiated areas. The neoblast apparently contributes, directly or indirectly, to the regeneration process of both non-irradiated and irradiated specimens. The neoblast probably forms some of the new mesodermal tissues.

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